



**PATENT APPLICATION**  
**Docket No. 356830.00300**

Former Docket No. 18810-80364

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

JULIA LJUBIMOVA et al.

Serial No.: 09/741,550

Filed: December 19, 2000

For: USING OVEREXPRESSION OF LAMININ  
ALPHA 4 SUBUNIT AS A DIAGNOSTIC  
AND PROGNOSTIC INDICATOR OF  
MALIGNANT TUMORS

Examiner: J. A. Goldberg

Art Unit: 1634

**DECLARATION OF JULIA LJUBIMOVA  
UNDER RULE 132 (37 CFR 1.132)**

I, JULIA LJUBIMOVA, declare as follows:

1. I am a named inventor on the present application and a citizen of the United States residing at 11419 Dona Pegita Dr., Studio City, California 91604

2. I obtained my M.D. degree from Kiev Medical University, Kiev, Ukraine in 1983 and a Ph.D. degree in Oncology-Pathology from the Kavetsky Institute for Oncology Problems, Academy of Sciences of Ukraine, Kiev, Ukraine. My thesis was entitled "Molecular peculiarities of DNA in malignant melanomas and benign nevi of human skin".

3. I have held positions of Staff Research Associate in the Department of Reproductive Medicine at the University of California San Diego and Research Scientist in the Department of Surgery and Neurosurgical Institute at Cedars-Sinai Medical Center, Los Angeles, California. I have also held appointments as an Adjunct Assistant Professor in the Department of Surgery at the University of California Los Angeles.

4. My current title is Director, Molecular Oncology Maxine Dunitz Neurosurgical Institute, Cedars-Sinai Medical Center. I also serve as a Clinical Assistant Professor in the Department of Neurosurgery at the University of California Irvine.

5. I have over twenty published scientific publications and have given numerous lectures and scientific presentations on cancer and related subjects.

6. I have a great deal of experience with malignancies both at an experimental and clinical level. During the studies that led to the above-named patent application I realized that  $\alpha 4$  laminin undoubtedly had significance in many, if not all, malignancies partially because of its relationship to vasculature in malignancies. Complete clinical studies are difficult and time consuming. However, prior to filing the above named application I was able to perform preliminary tests on malignant tumors not of brain origin. Although human breast cancer appears quite different from brain cancer, I discovered that like brain malignancies, malignant tumors of the breast overexpress  $\alpha 4$  laminin.

7. Since the filing of the present patent application I have expanded my observations on malignant breast tumors into a full study that parallels the instant patent application. As I show in the data presented below, the

results for malignant breast tumors are very similar to those for malignant brain tumors, thereby confirming the usefulness of the present invention in a variety of malignancies. I am also continuing to apply the inventive method to additional malignancies. My studies show that prostate malignancies, like those of the brain and the breast also overexpress  $\alpha 4$  laminin.

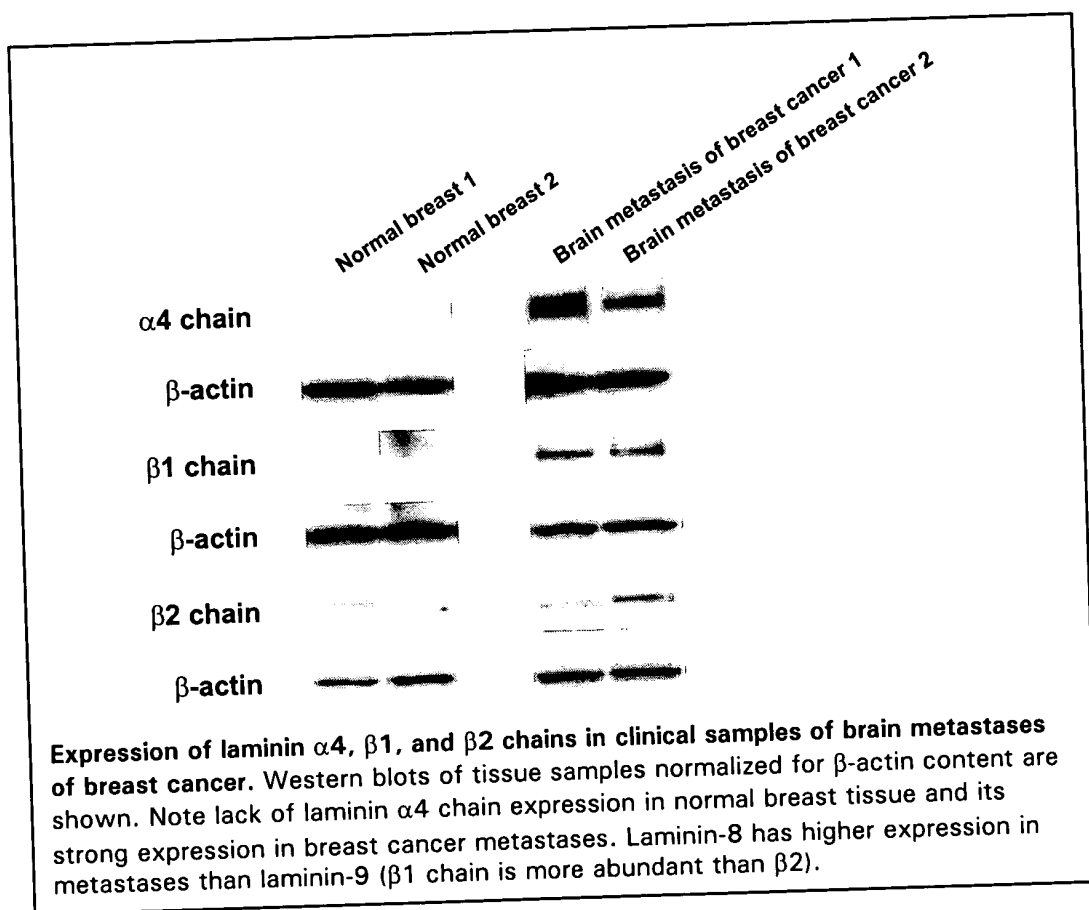
8. To demonstrate the present invention with breast malignancies 30 samples of human breast tissues were collected, 19 invasive breast carcinomas, 3 metastases of breast carcinoma to the brain, three breast carcinomas in situ and 5 normal breast tissues (Table 1). Sections were stained for immunofluorescence with antibodies to laminin  $\alpha 4$ ,  $\beta 1$ , and  $\beta 2$  chains, as well as to von Willebrand factor (to visualize endothelial cells) and to keratins 8/18 (to visualize epithelial cells). Tissue lysates were subjected to Western blot analysis using antibodies to laminin chains. Gel loading was normalized by  $\beta$ -actin staining.

**Table 1. Laminin expression in breast tissues by immunohistochemistry**

Histological diagnosis	Number of cases	Laminin-8	Laminin-9
Invasive carcinoma	19	16 (84%)	3 (16%)
Metastases of invasive carcinoma	3	3 (100%)	0
Noninvasive (in situ) carcinoma	3	1 (33%)	2 (66%)
Normal breast tissue	5	0	2 (40%)

9. By immunostaining, laminin-8 was absent in normal breast tissues. Weak expression of laminin-9 was seen in 2 of 5 cases. In carcinomas in situ, mostly laminin-9 was detected but one case also stained positive for laminin-

8 chains. Sixteen of 19 invasive breast carcinomas and all 3 metastases showed strong expression of laminin-8 chains. These laminin isoform expression patterns were very similar to those reported in the patent application for highly invasive brain tissues such as GBM, less invasive low-grade gliomas, and normal brain tissue. Western blot analysis (below) for laminin  $\alpha 4$ ,  $\beta 1$  and  $\beta 2$  chains confirmed the immunohistochemical data.

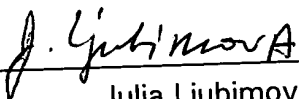


10. The Western blot results show that like brain malignancies, breast malignancies overexpress laminin  $\alpha 4$ . The normal breast tissue expresses a

much lower level of laminin  $\alpha 4$  than does the malignant tissue so that it is quite easy to differentiate normal from malignant tissue. This is an illustration of how simple it is to apply my inventive method to different malignancies, Although complex profiles of differential expression of multiple genes is contemplated by my invention, a relatively simple Western blot analysis readily demonstrates the laminin  $\alpha 4$ -malignancy relationship without need for complex experiments.

I hereby declare under penalty of perjury that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Executed this 17<sup>th</sup> day of March 2003 at Los Angeles, California .

  
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Julia Ljubimova